A stem cell screening platform for discovery of chondrogenic small molecules and their drug targets

PROGENITORTHERAPEUTICS regenerative drug discovery

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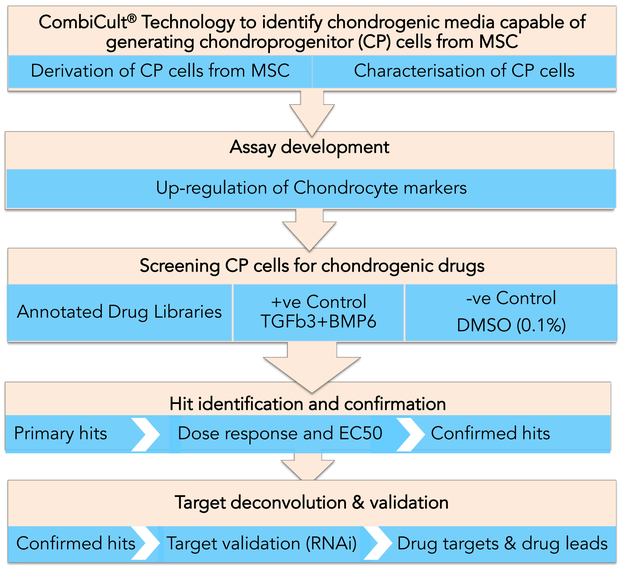
Plasticell

1) Summary

Drugs that promote chondrogenesis could be used to induce self-repair and regeneration of articular cartilage after traumatic injury or osteoarthritis (OA).

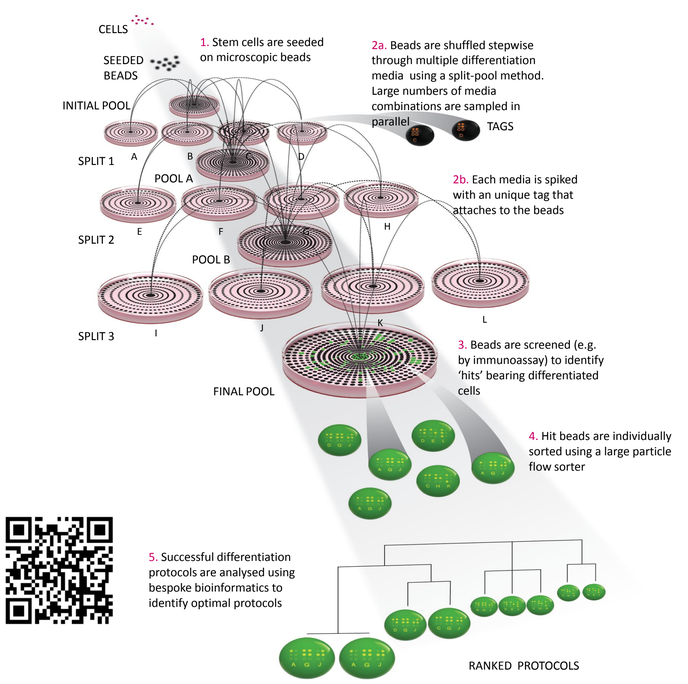
- Using Plasticell's bead-based combinatorial cell culture system CombiCult, we have developed serum-free chondrogenic media capable of generating chondroprogenitor cells from human MSC cultures. Chondroprogenitor cells derived in this way were further characterized by phenotype, stability and suitability for compound screening.
- We then carried out phenotypic drug screening on annotated chemical libraries consisting of ~7000 diverse set of compounds selected over a broad range of stem cell signalling and regulatory pathways. Through this approach, we discovered a number of hits that are capable of inducing chondrogenesis in vitro.
- We ranked our drug targets according to a selection criterion based on hit rate, EC50, maximum assay signal and biological relevance, then carried out RNAi knock down studies to validate targets.
- Using this approach we were able to identify and prioritise drug targets and potential drug leads for an OA program.
- We anticipate intra-articular administration of drugs developed from these hits will target the resident chondrogenic progenitor cell populations in the joint to promote repair and regeneration of damaged cartilage.

2) Project Overview



3) Combicult® Platform

Combinatorial cell culture (CombiCult®) is a bead-based, combinatorial technology specifically developed for discovery of novel stem cell differentiation protocols.



4) Screening of CP cells for Chondrogenic drugs

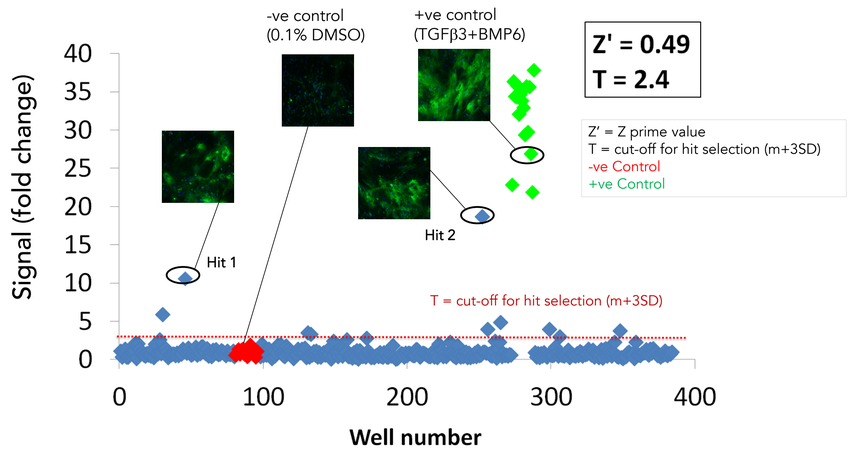


Fig 1. An example of screen and data analysis is shown. Our screening protocol consists of 3 stages; At stage 1, we plated a known number of cells into 384 well plates and cultured for three days. At stage 2, confluent MSC cells were primed with C121310 medium, resulting in CP cells that express CP1 and SOX9. At stage 3, CP cells were treated with compounds (10uM final) in screening media. Samples were immunostained for chondrogenic markers and analysed using the ArrayScan platform. The signal (% +ve cells) in each well was calculated and the final read out was expressed as a fold change compared to the 0.1% DMSO treated control. The cut-off for assigning hits was set at 3 standard deviations above the mean value of the DMSO control wells. Positive Control (TGFβ3+BMP6)

5) Hit confirmation, Putative Targets and Hit Rate

Dose response and EC50 of selected compounds

Fig 2. Dose response curve and EC50 of selected primary hit compounds are shown. 11 point serial dilution of each hit compound to generate a concentration response curve. Data was analysed using GraphPad Prism software with variable slope fit model. Log(compound) vs. Response is shown

Targets and hit rate

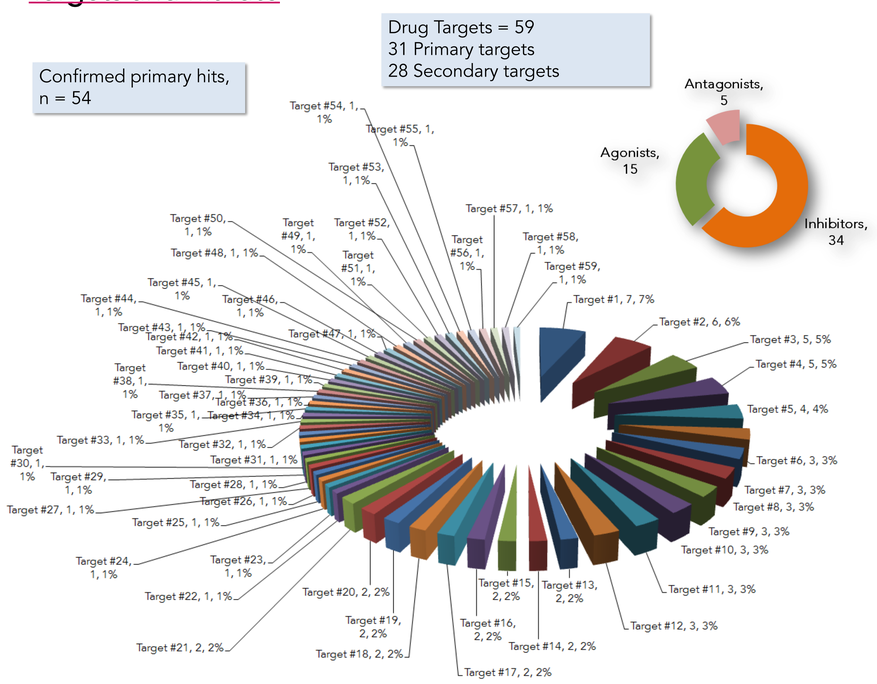


Fig 3. Summary of targets and hit rate from the chondrogenic drug screen: The pie chart represents hit frequency date for each of the 59 putative drug targets identified in this screen. There were 54 confirmed primary hits among the 7000 compounds screened. Hit annotation revealed one fifth of these targets were represented by ~50% of all the hits and the top target was being hit by 7 compounds represented by multiple chemotypes. Targets were ranked according to a selection criteria based on hit rate, potency or compounds EC50, maximum assay signal and biological relevance. A sub set of targets/compounds were selected for further validation to progress in a drug discovery programme.

6) Target Validation (RNAi)

Knockdown of inhibitor drug targets

- Lentiviral shRNA silencing of candidate targets, n =7 targets
- Aggrecan mRNA levels as output measure
- 5 out of 7 targets confirmed
- Data from target #2, #9 and #12 are shown below

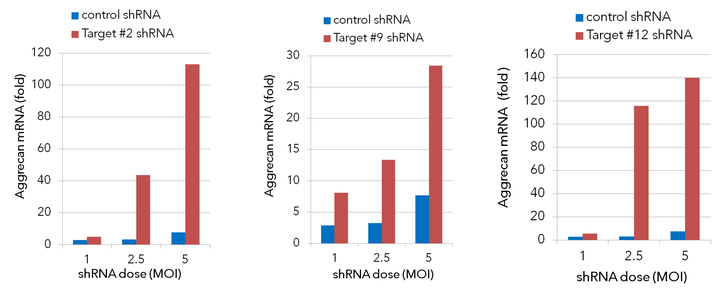


Fig 4. Quantitative mRNA analysis of aggrecan after 7 days of gene silencing of drug targets. Lentiviral mediated delivery of target-specific shRNA and control shRNA were tested at 3 different doses on CP cells in monolayer culture system. Aggrecan mRNA data are expressed as mean based on 2 to 5 samples per condition. Knock down of inhibitor targets led to constitutive induction in Aggrecan mRNA levels as analysed by RT-qPCR.

Knockdown of agonist drug targets (e.g. Target #4)

- Lentiviral shRNA silencing of agonist target (+/- agonist compound)
- knock down leads to → 65% reduction in chondrogenesis in the presence of agonist

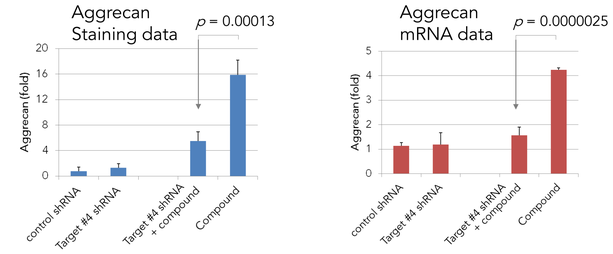


Fig 5. Aggrecan immunostaining data and mRNA levels analysed on day 7 after gene silencing by lentiviral mediated delivery of target-specific shRNA and control shRNA in monolayer cultures, +/- compounds. Aggrecan data are expressed as mean±SD (n=4 per condition). Reduction of an agonist's target impairs agonist-mediated aggrecan expression as analysed by immunocytochemistry and RT-qPCR.

7) Hit evaluation in 3D cultures

Effects on gene expression during chondrogenesis

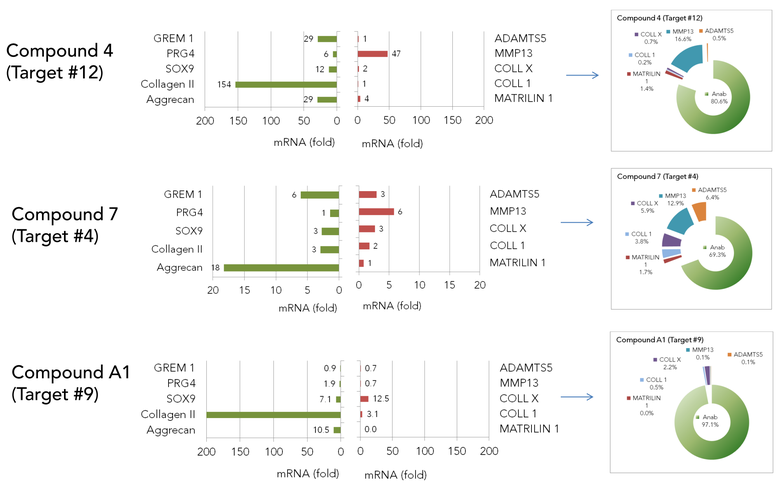


Fig 6. Quantitative mRNA analysis of compound-induced chondrogenesis of chondroprogenitor cells in 3D cultures on day 7. Relative mRNA fold change compared to the 0.1% DMSO treated control are shown. Pie charts plot the anabolic vs. catabolic (hypertrophy) balance of compound induced chondrogenesis as analysed by gene expression data.

8) Conclusions

- Derivation of chondroprogenitor cells in a 384 well format allows for high throughput phenotypic drug screen to discover chondrogenic drugs.
- A number of novel targets and compounds were identified for further follow up studies which will aid in the development of regenerative medicines for osteoarthritis treatment.

AKNOWLEDGEMENT:
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